**Tissue Engineering for In Vitro Analysis of Matrix Metalloproteinases in the Pathogenesis of Keloid Lesions**

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**IMPORTANCE** Keloid lesions form because of alterations in the mechanisms that govern cutaneous wound healing. Although matrix metalloproteinases (MMPs) have been implicated in keloid pathophysiology, many questions still remain about their involvement. Our incomplete understanding of keloid pathophysiology has led to high recurrence rates in current treatments. No reliable animal model is available for studying keloids.

**OBJECTIVE** To gain a better understanding of the disease mechanisms involved in keloid lesions in the hopes of identifying therapeutic options.

**DESIGN** Fibroblasts derived from keloid tissue were incorporated in either Matrigel or polyethylene glycol diacrylate mixed with type I collagen to create 3-dimensional models to investigate the role MMPs play in keloid formation. The MMP gene expressions were also compared between fibroblasts isolated from different sites within the same keloid lesion.

**SETTING** The Johns Hopkins School of Medicine, Baltimore, Maryland.

**PARTICIPANTS** Keloid fibroblasts were received from the Baylor College of Medicine, and additional keloid fibroblasts were enzymatically isolated from the dermal layer of lesions removed from consenting patients at The Johns Hopkins Hospital.

**RESULTS** In the Matrigel system, MMP9 and MMP13 were observed to be significantly upregulated in keloid fibroblasts. The addition of decorin resulted in a significant decrease of type I collagen and MMP1, MMP9, and MMP13 gene expressions from keloid fibroblasts. Higher MMP gene expressions were observed in fibroblasts isolated from the margins of the original keloid wound.

**CONCLUSIONS AND RELEVANCE** MMP9 and MMP13 are expressed significantly more in keloid-derived cells, thus making them 2 potential targets for disease modification. Molecules that target organization of the lesion’s matrix can be beneficial in downregulating increased markers during the disease. In addition, heterogeneity is observed with the varying MMP gene expressions from site-specific fibroblasts within the same keloid lesion.


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Keloids represent an extreme form of abnormal cutaneous wound healing that can result in both functionally restrictive and disfiguring scars. In normal wound healing, a series of physiologic responses results in the formation of a scar that is randomly disorganized and predominately composed of collagen deposition.1-2 Remodeling of the newly formed tissue affects the type of scar that forms. Keloid lesions are a form of abnormal wound healing whose molecular mechanism and pathogenesis are not well understood, thus making it a therapeutic challenge. In keloids, aberrant remodeling is thought to result in large quantities of collagen deposition after skin trauma in predisposed individuals. However, what distinguishes keloids is not only the abnormal quantity of collagen formation but also the physical extent of it. Clinically, keloids differ from hypertrophic scars in that they extend beyond the boundaries of the initial injury by invading surrounding healthy skin at the level of the dermis and fail to regress over time.3 An increase in inflammatory markers, such as transforming growth factor β1, and elevated levels of extracellular matrix (ECM) components, such as fibronectin and certain proteoglycans, are also associated with the formation of keloid lesions.4-9

Currently available treatments for keloid lesions include combinations of steroid injections, surgical removal, silicone
gels meant to hydrate keratinocytes with the aim of altering growth factor secretion, and radiation therapy. Although surgical removal of keloids can offer temporary cosmetic improvement, the surgical resection itself is often a trigger for the aberrant wound healing, thus risking more keloid formation, sometimes even extending into previously unaffected skin. In addition, steroid injections and radiation therapy are not free of potential adverse effects and are often also associated with recurrences.

Various hypotheses have been proposed to explain the pathogenesis of this disease, including abnormal regulation from the surrounding ECM proteins and irregularity in the enzymes that monitor ECM degradation and cellular migration. Matrix metalloproteinases (MMPs) are composed of a family of enzymes that are hypothesized to be involved in the keloid disease state because of their abnormal expression and activity, thus severely altering the remodeling process. Because of their important role in regulating connective tissue growth and wound healing, MMPs are considered potential targets in the treatment of keloids. However, studies so far have drawn conflicting conclusions regarding the upregulation or downregulation of these remodeling proteases in keloid formation. Common to all of these studies is the use of a monolayer cell culture. We believe that given the multidimensional nature of keloid physiology, a 3-dimensional cell culture system would better mimic the true environment of keloid formation and that, in fact, the use of monolayer cell culture may be contributing to this variation. Therefore, in this research, we applied tissue engineering strategies to create 3-dimensional in vitro disease models to analyze the changes in MMP gene expressions between normal and keloid fibroblasts. Two biomaterial systems were implemented for the models, and through one of the models, the therapeutic effects of an ECM modulator on the diseased fibroblast were also investigated, setting the precedence for future keloid studies with different ECM modulators. Lastly, the differences in remodeling markers from site-specific keloid fibroblasts were also analyzed to address the heterogeneity observed in the phenotypes of fibroblasts from keloid lesions.

Methods

Keloid Fibroblasts
Keloid fibroblasts were provided by Bindi Naik, MD, of Baylor College of Medicine and one of us (K.B.). Briefly, the isolation method involved rinsing keloid lesions thoroughly with phosphate-buffered saline solution containing penicillin, 100 U/mL, and streptomycin, 100 μg/mL (PBS-PS), on receipt. The epidermal layer was removed, whereas the dermis layer was minced before being placed in the collagenase digestion solution. The solution was composed of high-glucose Dulbecco modified Eagle medium (Invitrogen) with 10% fetal bovine serum (Hyclone), with a final concentration of 5 mg/mL of type 2 collagenase (Worthington) and 0.2 mg/mL of trypsin (Fluka). The minced explants were digested at 37°C with 5% carbon dioxide for 6 hours on an orbital shaker. The collagenase filtrate was then filtered with a 40-μm strainer and rinsed with PBS-PS 3 times before being plated in T-175 flasks with fibroblast media (FM). For encapsulation in biomaterials, all keloid fibroblasts were cultured in monolayer for 1 passage until confluency with FM. For lesion site–specific comparison, no monolayer expansion of keloid fibroblasts was performed.

Normal Skin Fibroblasts
As a control, normal human foreskin fibroblasts (HS27) were obtained from American Type Culture Collection and cultured in monolayer between 1 and 2 passages until confluency in FM before encapsulation in biomaterials. For lesion site–specific comparison, normal fibroblasts were isolated from a female patient who underwent an elective upper eyelid skin excision (blepharoplasty).

Medium Condition
The FM consisted of high-glucose Dulbecco modified Eagle medium (Invitrogen) with 10% fetal bovine serum and penicillin, 100 U/mL, and streptomycin, 100 μg/mL.

PEG-Coll In Vitro Experiment
The first in vitro design used polyethylene glycol diacrylate (PEGDA) and type I bovine collagen (PEG-Coll) as the polymer to encapsulate the fibroblasts (Figure 1A). Type I collagen was neutralized with 0.1N sodium hydroxide and then brought up to 2 mg/mL with PBS-PS. A 20% PEGDA solution in PBS-PS was mixed with the 2 mg/mL of type I collagen (BD Biosciences) to create constructs with the final concentrations of 10% PEGDA and 1 mg/mL of type I collagen. Fibroblasts, either keloid or normal (HS27), were mixed with the polymer solution at the concentration of 20 million cells/mL and then mixed with the photoinitiator Irgacure 2959 (Ciba) for a final concentration of 0.05% (wt/vol). Using UV light, the constructs of polymer-cell-photoinitiator were photopolymerized for 5 minutes at 3 mW/cm² and a 365-nm wavelength in cylindrical molds with a diameter of 5 mm. On encapsulation, the constructs were transferred to 24-well plates and cultured with 1.5 mL of FM for a total of 14 days. Constructs were harvested at days 2, 7, and 14 for data analysis. Medium was changed every 2 to 3 days until the time of harvest.

Matrigel In Vitro Experiment
The second in vitro model involved encapsulating fibroblasts in Matrigel (BD Bioscience). Cell density was 2 million cells per 100 μL of Matrigel, which was then incubated at 37°C for 30 minutes. Once gelation was achieved, the constructs were cultured in 24-well plates with 1.5 mL of FM per construct for a total of 21 days. Constructs were harvested at days 1, 7, and 21 for data analysis. In addition to the Matrigel constructs composed of keloid fibroblasts or HS27, a third group of constructs with encapsulated keloid fibroblasts were cultured with the addition of decorin, 5 μg/mL (Sigma), which was administered with every medium change, starting after 1 day of culture. Medium was changed every 2 to 3 days until the time of harvest.

Biochemical Analyses
Biochemical data included DNA content, proteoglycan content, and total collagen content. Harvested constructs were ly-
proline (Sigma-Aldrich) was dissolved in deionized water to react and demonstrated that keloid fibroblasts had increased type I collagen content. Polyethylene glycol diacrylate (PEGDA) was mixed with type I collagen fibrils before encapsulation of fibroblasts (HS27 and keloid fibroblasts) through UV photopolymerization. B, Reverse transcription–polymerase chain reaction demonstrated that keloid fibroblasts had increased type I collagen content.

Letters indicate statistical significance from HS27 at corresponding time point ($P < .05^a$ and $P < .001^b$); plus signs, statistical significance from day 2 of same cell type ($P < .05^c$, $P < .01^d$, and $P < .0001^e$). A, Schematic of type I bovine collagen in vitro model. Polyethylene glycol diacrylate (PEGDA) was mixed with type I collagen fibrils before encapsulation of fibroblasts (HS27 and keloid fibroblasts) through UV photopolymerization. B, Reverse transcription–polymerase chain reaction demonstrated that keloid fibroblasts had increased type I collagen content.

RNA Extraction and RT-PCR

Harvested constructs were homogenized in TRIzol Reagent (Invitrogen) in preparation for total RNA extraction. The protocol accompanying the reagent was followed for the extraction. Complementary DNA was then synthesized using the reverse transcriptase Superscript First-Strand Synthesis kit (Invitrogen). Reverse transcription–polymerase chain reaction (RT-PCR) was performed with Taq recombinant polymerase or with SYBR Green PCR Master Mix (Applied Biosystems) and conducted on the ABI Prism 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems). Amplicons of RT-PCR were mixed with a loading dye and then run on 2% agarose gels in Tris, acetic acid, and EDTA buffer within an electrophoresis box with an accompanying ladder dye. Pictures of the gels were taken after submerging them in diluted ethidium bromide and exposing them to UV light. The following genes were analyzed: type I collagen, MMP1, MMP2, MMP3, MMP9, MMP13, and MT1-MMP. All genes were normalized to the β-actin housekeeping gene. The MMP primers are listed in the article by Konttinen et al.14 Type I collagen primers are F-TGGCACCACACCTTCTACAT-2714 and R-GCACGCTTCTCCTTAATGTCACGC.

The hydroxyproline assay was used to measure total collagen content. Papain-digested samples were hydrolyzed for 18 hours at 115°C in 12N hydrochloride. After hydrolyzation, samples were mixed with methyl red, titrated with sodium hydroxide and hydrochloride, and then diluted with deionized water to reach the volume of 1 mL. Trans-4 hydroxy-L-proline (Sigma-Aldrich) was dissolved in deionized water to generate the standard curve. Both the standards and diluted samples were mixed with chloramine-tosylchloramide hydrate and p-dimethylaminobenzaldehyde and then incubated in 60°C for 30 minutes. Absorbance values were measured at 550 nm on a UV-Vis spectrophotometer, and the ratio of 1:10 hydroxyproline to collagen was used to calculate total collagen content.

The glycosaminoglycan (GAG) content was determined through the dimethylmethylen blue assay, which consisted of the dimethylmethylene blue dye mixed with standard and experimental samples. Varying concentrations of chondroitin sulfate C were used to generate the standard curve, whereas 50 μL of papain-digested samples was used to calculate the total GAG content per construct. The absorbance was measured at 525 nm on an UV-Vis spectrophotometer.

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Results

Verification of PEG-Col1 Model
Constructs were harvested at days 2, 7, and 14 for both normal (HS27) and keloid fibroblasts, and RT-PCR demonstrated higher expressions of type I collagen from keloid fibroblasts at all 3 time points (Figure 1B). This finding confirms that the altered phenotype that is associated with keloid disease is retained in this 3-dimensional PEG-Col1 model.

Matrix Production From Fibroblasts in the PEG-Col1 Model
Both HS27 and keloid fibroblasts demonstrated a decrease in DNA quantification during a span of 14 days in 3-dimensional culture (Figure 1C). However, at all time points, the GAG and total collagen production was significantly higher in keloid fibroblasts compared with normal fibroblasts (Figure 1, D and E). Specifically, there was a mean 2.02-fold increase in GAG per DNA and a 3.43-fold increase in total collagen per DNA in keloid fibroblasts.

MMP Gene Expression in Fibroblasts in the PEG-Col1 Model
The RT-PCR for MMP1, MMP2, MMP3, MMP9, MMP13, and MT1-MMP indicated that there were no significant differences between normal and keloid fibroblasts over time (Figure 2). MMP1, MMP2, MMP3, and MT1-MMP were consistently expressed in both fibroblast types at all time points. MMP9 and MMP13 decreased in expression in both HS27 and keloid fibroblasts as time increased.

Verification of the Matrigel Model
Constructs were harvested at days 1, 7, and 21, and as with the PEG-Col1 model, type I collagen was more strongly expressed by keloid fibroblasts than HS27 at all time points, indicating that the keloid disease phenotype is maintained in the Matrigel model (Figure 3, A and B).

Matrix Production From Fibroblasts in the Matrigel Model
As with the PEG-Col1 model, there was a decrease in DNA quantification as the time increased for both HS27 and keloid fibroblasts (Figure 3C). Both GAG and total collagen were produced in significantly greater amounts in keloid fibroblasts than in normal cells (Figure 3, D and E). Specifically, keloid fibroblasts produced 1.37-fold, 1.56-fold, and 2.08-fold more GAG per DNA than HS27 at days 1, 7, and 21, respectively. There was a time-dependent increase in GAG production in both normal and diseased fibroblasts; however, the fold changes from keloid fibroblasts were significantly higher than HS27. The trend for total collagen per DNA was similar to the GAG data. Keloid fibroblasts produced more matrix than HS27 at days 7 and 21, although this finding was not statistically significant. In addition, there was markedly more collagen at day 21 than day 1 from the keloid fibroblasts.

MMP Gene Expression in Fibroblasts in the Matrigel Model
MMP1, MMP2, MMP3, and MT1-MMP were consistently expressed in both HS27 and keloid fibroblasts in the Matrigel model at all time points (Figure 4). HS27 expressed little to no MMP9 and MMP13, whereas keloid fibroblasts increased expression of both MMP genes over time.

Effects of Decorin on Matrix Production in Keloid Fibroblasts in the Matrigel Model
Decorin was exogenously added into the medium of keloid fibroblasts encapsulated in Matrigel from day 1 to day 21. At days 7 and 21, constructs were harvested after treatment with decorin. There was a decrease in DNA quantification as time increased, whether decorin was added or not (Figure 5A). Dimethylmethylene blue assay demonstrated that, at day 21, the quantities of GAG per DNA were the same at day 7 and day 21 when keloid fibroblasts were exposed to decorin (Figure 5B). This resulted in significantly less GAG per DNA at day 21 in keloid fibroblasts that were not exposed to decorin. Quantification of total collagen per DNA also demonstrated a similar trend as the GAG data, although the finding was not statistically significant (Figure 5C).

Effects of Decorin on Gene Expression From Keloid Fibroblasts
Type I collagen gene expression was significantly downregulated at day 21, when decorin was administered to keloid fibroblasts (Figure 5D). In addition, MMP1 and MMP13 were also significantly decreased at day 21 when keloid fibroblasts were exposed to decorin.

Statistical Analysis
All analyses were performed in triplicate and analyzed with the t test for pairwise comparison. Statistical significance was set at P < .05.
treated with decorin. MMP9 did not change significantly in gene expression between day 7 and day 21 when keloid fibroblasts were treated with decorin, and this differed from untreated fibroblasts for which an increase in MMP9 was observed by day 21.

**MMP Gene Expression From Specific Sites of Keloid Lesion**

Two keloid lesions were received from one patient; one lesion was removed from the shoulder and the other from the ear. Because of the small size of the auricle lesion, the enzymatic isolation resulted in a mix of fibroblasts that were not separated by tissue depth and thus were labeled as “mixed auricle.” The lesion from the shoulder was separated into 4 different sites: superficial side fibroblasts, superficial center fibroblasts, deep center fibroblasts, and keratinocytes (Figure 6A). The MMP gene expressions were analyzed among the different cell populations, both shoulder and auricle, and compared with normal fibroblasts that were isolated from the skin of a patient who underwent eyelid skin excision.

Normal fibroblasts demonstrated little to no expression of all MMP genes tested (Figure 6B). In comparing the fibroblasts isolated from the shoulder, the superficial side and deep center had the highest expressions of MMP1, MMP2, MMP3, MMP9, and MT1-MMP. The superficial center fibroblasts had little to no expression of those MMP genes. All fibroblasts from the shoulder had little to no expression of MMP13. The keratinocytes also expressed MMP2 and MMP3 with comparable intensities to superficial center fibroblasts and slightly higher expressions of MMP1 and MMP9 than the superficial center fibroblasts. The mixed auricle fibroblasts had high expressions of all tested MMP genes, including MMP13.

**Discussion**

Keloids remain a clinical challenge and a source of significant psychological distress to patients who can have grossly dis-
native 3-dimensional environment and observed how of this experiment, we studied keloid fibroblasts in their more potentially explain this discrepancy. Therefore, in the first part of the analysis, we focused on implementing analysis of keloid disease, we focused on implementing what the fibroblasts sense in their native environment within connective tissue, in addition to promoting cellular adhesion. Although in vitro encapsulation of both normal and diseased fibroblasts resulted in type I collagen gene expression at all 3 time points of harvest, keloid fibroblasts expressed significantly more type I collagen than HS27, thus indicating that the diseased fibroblasts in this 3-dimensional model maintain their diseased phenotype in vitro. Biochemical analysis also indicated that at all 3 time points diseased fibroblasts had higher rates of ECM production than normal fibroblasts. Therefore, keloid fibroblasts in this 3-dimensional model still exhibited their diseased characteristics not only in gene expression but also in actual matrix synthesis.

Interestingly, not a lot of differences were found in MMP gene expressions between normal and keloid fibroblasts when cultured in the PEG-Col1 3-dimensional model. In general, most of the analyzed MMP genes were expressed consistently through all time points for both fibroblast types. However, both MMP9 (gelatinase B) and MMP13 (collagenase 3) were strongly expressed at day 2 for both cell types and then downregulated as time increased. Although this finding suggested that MMP9 and MMP13 are potential therapeutic targets, more research is needed to confirm these results.

In creating the next in vitro 3-dimensional model for further analysis of keloid disease, we focused on implementing a material that would better mimic the diseased environment. Matrigel is a heterogeneous basement membrane protein mixture secreted by cells from mouse sarcoma and com-

Letters indicate statistical significance from HS27 at corresponding time point (P<.05); plus signs, statistical significance from day 7 of same cell type (P<.001). A, DNA quantification normalized to respective dry weights indicated decrease in both conditions as time increased. B, Glycosaminoglycan (GAG) content normalized to DNA demonstrated that the presence of decorin prevented an increase in the matrix component production. C, Total collagen content normalized to DNA demonstrated a similar trend as the GAG data, although not statistically significant. D, Reverse transcription–polymerase chain reaction of type I collagen and MMP genes that were affected by the presence of decorin administered to keloid fibroblasts. Significant downregulations in type I collagen, MMP1, and MMP13 were observed at day 21, whereas MMP9 retained the same expression as day 7 and was prevented from being upregulated.
commercialized by BD Bioscience. It resembles the ECM of various connective tissues, and it is composed of different matrix components, such as gelatin, fibronectin, laminin, type IV collagen, and various growth factors. Matrigel has been commonly used for the study of cellular attachment and differentiation and especially the study of tumor cell invasion. In addition, connective tissue diseases have been associated with keloids. Therefore, Matrigel was used in our second 3-dimensional in vitro model to encapsulate keloid fibroblasts for further characterization of the diseased cells.

Similar to the PEG-Col1 model, type I collagen was expressed strongly by keloid fibroblasts and weakly by normal fibroblasts when the cells were cultured in Matrigel. Keloid fibroblasts also produced more GAG and total collagen per DNA than HS27, thus demonstrating that culturing the diseased fibroblasts in Matrigel maintained their excessive synthetic activity state. Interestingly, more significant differences were observed with GAG production than collagen production, with higher-fold changes in GAG per DNA from the diseased fibroblasts as time increased. The literature has cited higher concentrations of proteoglycans from healing wounds on animal models and different types of tumors removed from patients and overexpression of various proteoglycans, such as chondroitin sulfate, versican, and dermatan sulfate, through microarray. Therefore, the increase in GAG production per DNA from the keloid fibroblasts in the Matrigel model is in accord with the benign tumorlike characteristic of keloids. In addition, the continuous increase in GAG production from the keloid fibroblasts suggests that the diseased cells have an abnormal phenotype that retains them in a perpetual healing stage, which could be the cause of the excessive fibroproliferation of the lesions.

The MMP gene analysis from the Matrigel model demonstrated that MMP1, MMP2, MMP3, and MT1-MMP were generally consistently expressed from both HS27 and keloid fibroblasts at all time points, similar to the trends observed with the PEG-Col1 model. However, significant differences were observed in MMP9 and MMP13. Although there was little to no expression of either MMP gene from the normal fibroblasts at any time point, keloid fibroblasts expressed more MMP9 and MMP13 as time increased. Therefore, the data from the Matrigel model suggest that the gelatinase and collagenase are 2 main players in the progression of keloid lesions through an upregulation of MMP gene expression that potentially stimulate more remodeling of the wound and thus result in excessive tissue formation. In addition, because both MMP genes were upregulated in expression as time increased, this finding indicates that the 2 proteases potentially function in parallel within keloid lesions.

The difference observed in the MMP gene expression changes between the 2 different biomaterial models supports the hypothesis that the surrounding ECM has an important role in the keloid pathogenesis. Another hallmark of the keloid ECM is the lack of structural organization in the scar formation. Therefore, we hypothesized that treating the diseased cells with a molecule that promotes orderly ECM arrangement could be a therapeutic treatment for the disease. Decorin is a form of GAG that functions to maintain skin integrity and structural organization by binding to collagen fibrils. Yeo et al observed that healing skin has significantly less decorin than normal skin and that connective tissue from normal human breast stained strongly for decorin, whereas Reed and Iozzo observed irregular collagen fibrils in decorin knockout mice. In our study, decorin was administered exogenously to keloid fibroblasts with every medium change in an attempt to produce a more organized ECM rather than the random arrangement that is characteristic of keloid ECM. After 21 days, although untreated keloid fibroblasts increased GAG production from day 7, those that were treated with decorin maintained the same content of GAG per DNA, suggesting that the presence of decorin prevented the diseased fibroblasts from producing more GAG. In addition, a similar trend was observed when quantifying total collagen per DNA at day 21 when

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Figure 6. Site-Specific Gene Expressions

A, Different sites from which keloid fibroblasts were isolated from the shoulder lesion for comparison of MMP gene expressions among the different sites: i, superficial side; ii, superficial center; iii, deep center; and iv, keratinocytes. Keratinocytes were also isolated from the shoulder lesion. B, Reverse transcription-polymerase chain reaction of MMP gene expressions from primary fibroblasts isolated from different sites of a keloid lesion and compared with the corresponding keratinocytes that lined the epidermis of the lesion, a mixed fibroblast population from the ear, and normal fibroblasts isolated from an eyebrow lift. Fibroblasts from regions closest to the margins of the original wound and the excision site (ie, superficial side and deep center) had higher expressions of MMP1, MMP2, MMP3, and MMP9 compared with those farthest away from the wound boundaries (superficial center). Keratinocytes also expressed the same 4 MMP genes, although at lower intensities, when compared with the superficial side and deep center. The mixed keloid fibroblast had high expressions of MMP genes, whereas normal fibroblasts demonstrated little to no expression of the enzymes.
untreated keloid fibroblasts produced more of the matrix than treated fibroblasts. We hypothesize that the presence of decorin bound to the collagen fibrils in the surrounding environment of the keloid fibroblasts, which resulted in a more organized arrangement of the ECM and thus downregulated ECM production.

In addition, type I collagen and MMP gene expressions were affected by the presence of decorin. Type I collagen, MMP1, and MMP3 were significantly downregulated in expression at day 21 after continuous treatment with decorin, whereas MMP9 expression was maintained from day 7 to day 21 without the increase that was observed from untreated fibroblasts. This finding suggests that an increase in MMP expression is associated with the growth of a keloid lesion and that the presence of a GAG that enhances structural organization could downregulate the expressions of the MMP genes. Furthermore, we observed that an increased expression of MMP genes coincides with excessive ECM deposition, which also supports the idea that targeting MMP genes can therapeutically regulate ECM production.

Heterogeneity is another characteristic of keloid disease that renders it a challenge to fully elucidate the mechanisms that govern its pathogenesis. Specifically, differences are observed in cell behavior and phenotype, depending on the region of keloids from which the cells were derived. Lu et al. demonstrated that most fibroblasts derived from the peripheral region of keloids were in a proliferative state, whereas fibroblasts isolated from the central region of keloids were generally in the quiescent phase. A study by Sayah et al. demonstrated that the apoptotic indices were different between peripheral fibroblasts and central fibroblasts. A 2008 published work on monolayer culture by Seifert et al. suggested that site specificity of fibroblast isolation can affect how gene expressions are observed. The heterogeneity demonstrated by these works on site specificity could also potentially explain the varying results that have been observed on differing MMP gene expressions, in addition to the monolayer cultures. Therefore, to study the changes in MMP gene expression due to site specificity, we isolated primary fibroblasts from different regions of a keloid lesion and compared the MMP gene expressions to a mixed keloid cell population and to a normal cell population. Fibroblasts were isolated from the dermis of a shoulder lesion and separated into 3 regions as depicted in Figure 6A. There were higher MMP gene expressions from the regions of the lesion that were closer to the margins of the original wound (ie, superficial side) and the region within the deep center of the keloid. The fibroblasts from the superficial periphery and the deep center strongly expressed MMP1, MMP2, MMP3, and MMP9 when compared with the superficial center fibroblasts. The cells isolated from the ear consisted of a mixed fibroblast population and strongly expressed all tested MMP genes, whereas fibroblasts from normal skin generated very little to no expression. These data suggest that fibroblasts that are closer to the margins of the original wound, where active cellular migration and reepithelialization occur, have higher MMP gene expression, which could have a role in stimulating more tissue remodeling, thus resulting in excessive growth. In addition, literature has cited that interactions between keratinocytes and fibroblasts are important in the process of wound healing, and the presence of keloid keratinocytes can increase fibroblast proliferation and the secretion of soluble collagen types I and III. Therefore, we also compared differences in the protease gene expression between keloid fibroblasts and keloid keratinocytes derived from the shoulder lesion. However, the keratinocytes did not express the MMP genes with as much intensity as some of the fibroblast population, thus indicating that the fibroblasts within the dermis of the keloid lesion are most likely the predominant contributors in higher MMP gene expressions.

In conclusion, our in vitro data demonstrate that MMP9 and MMP3 are 2 potential targets in therapeutically treating keloid lesions. Their concurrent expressions from the Matrigel 3-dimensional disease model suggest that they enhance the expressions of each other, thus promoting the growth of keloid lesions. Through manipulating the ECM, we observed that molecules that target organization of the lesion’s matrix can possibly be beneficial in downregulating increased markers during the disease. Site specificity of fibroblasts from keloid lesions have demonstrated different intensities of MMP gene expressions, thus further supporting the heterogeneity that is observed in keloid fibroblast phenotype. These studies set the precedent for future tissue engineering studies to better elucidate the keloid pathogenesis.